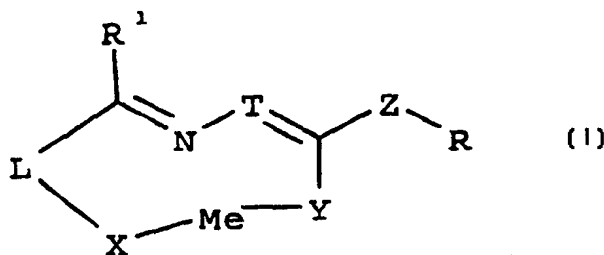




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## (57) Abstract

The present invention is directed to the use of metal complexes of general formula (I) with Schiff base ligands, which contain sulfur, nitrogen and oxygen as donor atoms, have both an agonistic and synergistic effect on the TPO receptor, in the treatment of diseases or disorders where thrombopoietin or another peptide/protein binding to the mp1 receptor is used as therapeutic agent, particularly in the treatment of thrombopenias and anemias, and drugs containing same, wherein L, X, Me, Y, Z, R, T, and R<sup>1</sup> have the above-specified meanings.

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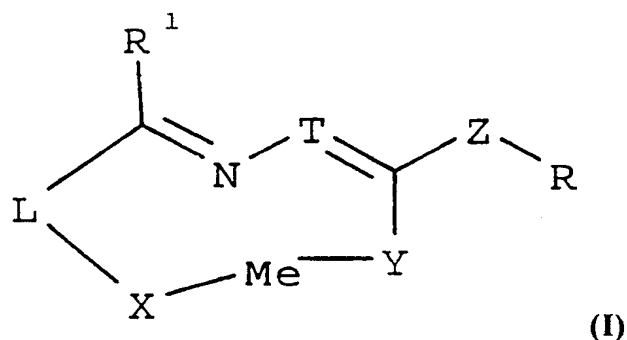
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**MPL-RECEPTOR LIGANDS, PROCESS FOR THEIR PREPARATION, MEDICAMENTS CONTAINING THEM AND THEIR USE FOR THE TREATMENT AND PREVENTION OF THROMBOCYTOPAENIA AND ANAEMIA**

The present invention is directed to metal complexes with Schiff base ligands, which contain sulfur, nitrogen or oxygen as donor atoms, have an agonistic and/or synergistic effect on the TPO receptor, methods of preparing same, and drugs containing same.

The invention relates to metal complexes of general formula I

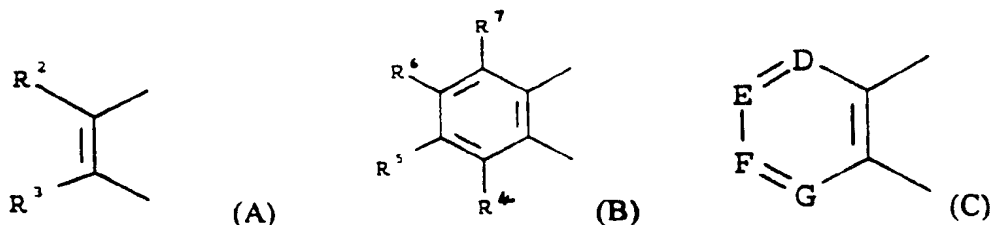


wherein

- Me represents cobalt, copper, nickel or zinc, which may optionally form a bond to N;
- X represents sulfur, oxygen or an amino group which may be substituted by C<sub>1</sub>-C<sub>10</sub> alkyl or benzyl;
- Y represents oxygen, sulfur or an amino group which may be substituted by C<sub>1</sub>-C<sub>10</sub> alkyl, benzyl or phenyl;
- T represents nitrogen or CR<sup>12</sup>, wherein R<sup>12</sup> may be hydrogen, C<sub>1</sub>-C<sub>10</sub> alkyl, benzyl or phenyl;
- Z represents oxygen, NH or a bond;
- R represents hydrogen, phenyl which may optionally have one or more substitutions, or pyridyl;

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- $R^1$  represents hydrogen,  $C_1$ - $C_{10}$  alkyl, phenyl which may optionally have one or more substitutions, a carboxyl or  $C_1$ - $C_{10}$  alkoxy carbonyl group;
- L represents an ethylene group (A), an aromatic ring (B), or a heterocyclic ring (C):



wherein

$R^2$ ,  $R^3$  independently represent hydrogen,  $C_1$ - $C_{10}$  alkyl, phenyl, carboxyl,  $C_1$ - $C_{10}$  alkoxy carbonyl, or aminocarbonyl;

$R^4$ ,  $R^5$ ,  $R^6$ , and  $R^7$  independently represent hydrogen, chlorine, bromine, iodine, fluorine, trifluoromethyl, cyano,  $SO_3H$ ,  $SO_3Na$ ,  $-SO-R^9$ ,  $-SO_2-R^9$ , nitro, phenyl which may optionally be substituted,  $C_1$ - $C_{10}$  alkyl,  $C_1$ - $C_{10}$  alkoxy,  $C_1$ - $C_{10}$  acyloxy, aralkoxy,  $-CO-R^9$ ,  $NR^{10}R^{11}$ , hydroxy, or cycloalkyl;

wherein

$R^9$  may be hydroxy,  $C_1$ - $C_{10}$  alkyl, phenyl, amino, mono- or dialkylamino;

$R^{10}$  and  $R^{11}$  independently represent hydrogen,  $C_1$ - $C_{10}$  alkyl, phenyl, benzyl, or  $C_1$ - $C_{10}$  acyl;

$R^5$ ,  $R^6$  together represent the  $-CH=CH-CH=CH-$  group;

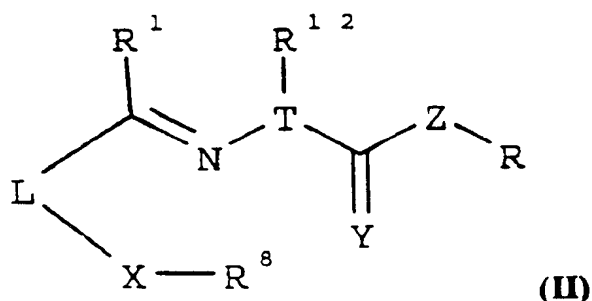
$R^1$ ,  $R^7$  together may form a carbocyclic saturated or unsaturated ring system having 5-14 C atoms, which may optionally have one or more substitutions by halogen, nitro, hydroxy,  $C_1$ - $C_{10}$  alkyl,  $C_1$ - $C_{10}$  alkoxy,  $C_1$ - $C_{10}$  alkoxy carbonyl, amino, sulfonyl, sulfinyl, mercapto,  $C_1$ - $C_{10}$  alkylmercapto, mono- or di- $C_1$ - $C_{10}$ -alkylamino;

D, E, F, G independently represent  $CR^4$  or N, where either the symbols  $D=E$  or  $F=G$  may also represent oxygen,

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sulfur or  $\text{NR}^{10}$ , or the symbols  $\text{D}=\text{E}$ ,  $\text{E}=\text{F}$ ,  $\text{F}=\text{G}$  may be components of another fused ring system; and their optically active forms, racemates, tautomers, diastereomeric mixtures, as well as the physiologically tolerable salts and prodrugs of these compounds.

The invention is also directed to compounds of general formula II

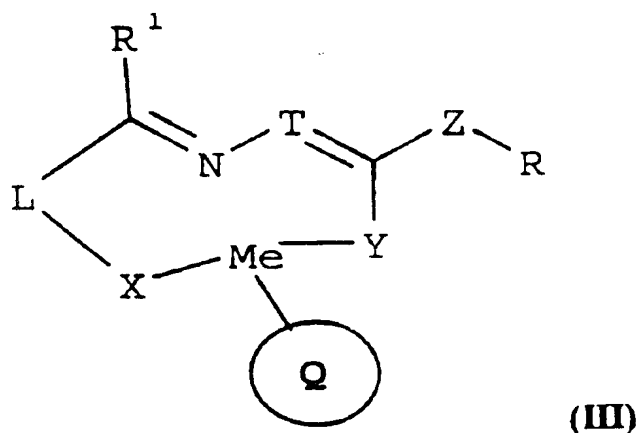


wherein

$\text{R}$ ,  $\text{R}^1$ ,  $\text{R}^{12}$ ,  $\text{Y}$ ,  $\text{Z}$ ,  $\text{L}$ , and  $\text{T}$  have the meanings specified for formula I,  $\text{X}$  represents sulfur, oxygen or an amino group which may be substituted by  $\text{C}_1$ - $\text{C}_{10}$  alkyl or benzyl, and  $\text{R}^8$  represents hydrogen, benzyl, acetyl or  $\text{C}_1$ - $\text{C}_{10}$  alkyl, and their optically active forms, racemates, tautomers, diastereomeric mixtures, as well as the physiologically tolerable salts and prodrugs.

In addition, the invention is directed to compounds of general formula III

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wherein

R, R<sup>1</sup>, L, Me, T, X, Y, and Z have the meanings specified for formula I, Q represents tetrahydrofuran, dimethyl sulfoxide, dimethylformamide, ammonia, a primary, secondary or tertiary amine, pyridine, a trialkylphosphine or triphenylphosphine, 1-Me-3,4-dihydroisoquinoline, 1,3,3-trimethyl-4-hydroisoquinoline, and their optically active forms, racemates, tautomers, diastereomeric mixtures, as well as the physiologically tolerable salts and prodrugs.

In addition, Q may be a compound of general formula II, so that a complex is formed which contains metal ion and ligand at a ratio of 1:2. Q may also be a compound of general formula I, so that a dimeric complex is formed which contains metal ion and ligand at a ratio of 1:1.

The invention also relates to methods of preparing the above compounds, drugs containing these compounds, and the use of these compounds in the production of drugs.

The compounds of general formula I, II or III have valuable pharmacological properties. They act as thrombopoietin agonists and/or synergists and thus, are suitable in the treatment of diseases where *inter alia*, thrombopoietin or other proteins/peptides binding to the mpl receptor (thrombo-

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poietin receptor) are used as therapeutic agents. In particular, they are suitable in the treatment of hematopoietic disorders, e.g., in the therapy of thrombopenias and anemias, e.g., following chemo- or radiotherapy or bone marrow transplantation, and in the mobilization of stem and progenitor cells. Also, they may be used in the *in vivo* and *in vitro* expansion of stem cells to regenerate the hematopoietic system and to provide modified stem cells for gene-therapeutic uses. In the following, the term "TPO" will be used for all proteins/peptides binding to mpl.

Amongst the various cells of blood which, having a lifetime ranging from only a few hours to up to 20 days, must constantly be regenerated, the megakaryocytes produced by progenitor cells are an important group. Megakaryocyte growth and development is controlled by hematopoietic growth factors. Thus, on the one hand, they effect expansion of the megakaryocyte progenitors (megakaryopoiesis) and, on the other hand, induce megakaryocyte maturing up to formation of thrombocytes (thrombopoiesis). Thrombocytes, also referred to as blood platelets, are small cells which contribute to blood clotting, and close wounds as a result of their ability of aggregating. Following fragmentation of the cytoplasm, megakaryocytes release blood platelets into the vascular space. In a healthy person, 3-10 billion thrombocytes are produced by the blood-generating cells of the bone marrow.

Chemo- or radiotherapy in the treatment of cancer, various infectious diseases, leukemia or aplastic anemia may result in a life-threatening damage to the blood cells. Likewise, large amounts of hematopoietic cells must be resynthesized subsequent to a bone marrow transplantation, and in some rare cases, congenital defects are present as cause for a decreased platelet number.

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Referring to the U.S.A. alone, more than 250,000 patients undergo chemotherapy, at least one third of them become diseased with thrombocytopenia and therefore, approximately 10 million thrombocyte transfusions are required. To this end, however, an enormous amount of stored blood is needed and, in addition, problems arise such as alloimmunization, possible transmission of viral and bacterial infections, as well as congestive heart failure.

The factor which is responsible for the humoral control of megakaryocyte growth and platelet generation, namely, TPO (thrombopoietin, also referred to as M-GDF (megakaryocyte growth and development factor)) was first isolated in 1994 by various groups [1-3]. The physiological TPO binding site is the mpl receptor which, for example, is present on CD34<sup>+</sup> cells, megakaryocytes and platelets [4].

In addition to its effect on megakaryopoiesis, TPO also stimulates erythropoiesis [5] and therefore, also increases formation of erythrocytes in myelo-suppressed, irradiated mice which had been treated with a chemotherapeutic agent. Moreover, it has been possible to achieve a raise in neutrophils [5]. Similarly as in animal models, TPO effects an increase of blood platelets in tumor patients with thrombopenia [6,7] and exhibits good tolerability (WO-A-96/15758, WO-A-97/16535).

Therefore, by using TPO alone or in combined action with EPO and G-CSF which represent the stimulating factors in the formation of erythrocytes and granulocytes, respectively, it should be possible to accomplish higher and more frequent doses in radio- and/or chemotherapy and consequently, enable a more effective cancer therapy.

However, treatment using TPO human protein involves a number of drawbacks:



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Being a recombinant protein, it is extremely expensive, it has to be administered on the parenteral route due to lacking oral bioavailability, and it is liable to rapid degradation by proteases to form inactive fragments. WO-A-96/40750 describes peptides having TPO-agonistic activity, but in this case as well, there is the same problem of lacking oral bioavailability and sensitivity to proteases, making it necessary to administer these substances by injection or infusion.

Low molecular weight substances of general formula I, II or III surprisingly show TPO-agonistic and synergistic activity that has been unknown to date and therefore, they are valuable drugs.

Surprisingly, they also stimulate the *in vitro* formation of hematopoietic cells and are capable of *in vivo* increasing the number of stem cells in peripheral blood for both autologous and allogenic blood cell donation. Using the compounds according to the invention, it is also possible to expand the platelets *in vivo* for an autologous blood cell donation.

In the compounds of formula I, II or III, C<sub>1</sub>-C<sub>10</sub> alkyl in all cases represents a straight or branched C<sub>1</sub>-C<sub>10</sub> chain which may optionally have one or more substitutions by C<sub>1</sub>-C<sub>10</sub> alkyl or hydroxy, with methyl, ethyl, propyl, i-propyl, n-butyl, tert-butyl, pentyl, hexyl, heptyl, octyl, nonyl, or decyl groups being preferred.

In all cases, the C<sub>1</sub>-C<sub>10</sub> alkoxy groups in the compounds of formula I, II or III contain straight or branched C<sub>1</sub>-C<sub>10</sub> alkyl chains, with methoxy, ethoxy, n-propyloxy, i-propyloxy, n-butyloxy, tert-butyloxy, pentyloxy, hexyloxy, heptyloxy, octyloxy, nonyloxy, or decyloxy groups being preferred.

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Preferably, the C<sub>1</sub>-C<sub>10</sub> acyl residue is understood to be a formyl, acetyl, propanoyl, butanoyl, pentanoyl, hexanoyl, heptanoyl, octanoyl, nonanoyl, or decanoyl residue.

The aralkyloxy groups contain a phenyl group linked with a C<sub>1</sub>-C<sub>10</sub> alkoxy group, the benzyloxy, phenylmethoxy and phenylethoxy groups being preferred.

The carbocyclic saturated or unsaturated ring systems having 5-14 C atoms, which are formed by R<sup>1</sup> and R<sup>7</sup> together and may optionally have one or more substitutions, are understood to be cyclopentane, cyclohexane or indane, for example, with cyclopentane and indane being preferred.

If R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, or R<sup>7</sup> in the compounds of general formula I, II or III is a cycloalkyl group, it is understood to be a ring having from three to six carbon atoms.

The substituents of a carbo- or heterocyclic saturated or unsaturated ring system are understood to be halogen, nitro, cyano, hydroxy, C<sub>1</sub>-C<sub>10</sub> alkyl, C<sub>1</sub>-C<sub>10</sub> alkoxy, C<sub>1</sub>-C<sub>10</sub> alkoxycarbonyl, amino, C<sub>1</sub>-C<sub>10</sub> alkylsulfonyl, C<sub>1</sub>-C<sub>10</sub> alkylsulfinyl, mercapto, C<sub>1</sub>-C<sub>10</sub> alkylmercapto, mono- or di-C<sub>1</sub>-C<sub>10</sub>-alkylamino, or trifluoromethyl.

If one of the symbols D=E or F=G represents oxygen, sulfur or NR<sup>10</sup>, formula C represents a five-membered heterocyclic ring such as furan, thiophene, pyrrole, imidazole, oxazole, thiazole, pyrazole, or isoxazole. If one of the symbols D=E or E-F or F=G is part of another fused ring system, D and E or E and F or F and G in this case are linked by an appropriate chain. Such a chain which may optionally be substituted by C<sub>1</sub>-C<sub>10</sub> alkyl or hydroxy is exemplified by the following: -CH=CH-CH=CH-, -(CH<sub>2</sub>)<sub>3</sub>-, -(CH<sub>2</sub>)<sub>4</sub>-, -CH=CH-S-, -CH=CH-O-, -CH=CH-NH-, -CH=CH-CH=N-, -CH=N-CH=CH-, -(CH<sub>2</sub>)<sub>3</sub>-CO-.

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Those compounds of general formula I, II or III are particularly preferred wherein Me represents nickel,  $R^1$  is hydrogen, L represents the group (B), Q is ammonia in the case of formula III, and  $R^4$ ,  $R^5$ ,  $R^6$ , and  $R^7$  independently represent hydrogen,  $C_1$ - $C_{10}$  alkoxy,  $C_1$ - $C_{10}$  acylamino, benzyl-oxy,  $C_1$ - $C_{10}$  monoalkylamino, amino, di- $C_1$ - $C_{10}$ -alkylamino, or halogen,  $SO_3Na$ , or  $SO_3H$ , or  $R^4$  and  $R^6$  at the same time represent halogen; it is particularly preferred that halogen represents chlorine or bromine, X is oxygen, T is nitrogen, Z is NH, and Y represents oxygen or sulfur.

Prodrugs are understood to be compounds which are metabolized *in vivo* to give compounds of general formula I, II or III.

Examples of physiologically usable salts of the compound of formula I are salts with physiologically tolerable mineral acids such as hydrochloric acid, sulfuric acid, sulfurous acid or phosphoric acid, or with organic acids such as methanesulfonic acid, p-toluenesulfonic acid, acetic acid, trifluoroacetic acid, citric acid, fumaric acid, maleic acid, tartaric acid, succinic acid, or salicylic acid. Compounds of formula I having a free carboxyl group may also form salts with physiologically tolerable bases. Examples of these salts are alkali metal, alkaline earth metal, ammonium, and alkylammonium salts, such as sodium, potassium, calcium, or tetramethylammonium salts.

The pure enantiomers of the compounds of formula I, II or III are obtained either by resolving the racemates (via salt formation with optically active acids or bases) or by using optically active starting materials in the synthesis.

The preparation of compounds of general formula I and II, wherein  $R^6$  represents bromine, hydrogen or a nitro group, is described in Zh. Neorg. Khim. 32, 1158 (1987). This type

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of compound was shown to have *in vitro* antibacterial activity (Zh. Neorg. Khim. 24, 40 (1990)). Compounds of general formula I or II having other substituents are prepared in an analogous manner or may subsequently be converted to compounds of general formula I or II by exchanging substituents. The synthesis and physical properties of the substances of general formula I wherein Z is a bond and R represents 2-OH-C<sub>6</sub>H<sub>4</sub> are reported in Zh. Obshch. Khim. 60, 2348 (1990). The compounds of general formula II are precursors in the synthesis of compounds of general formula I.

Zh. Obshch. Khim. 60, 2549 (1990) describes the preparation of compounds of general formula III, wherein Q represents pyridine, aniline or an aliphatic amine. Compounds of general formula III may be used as catalysts in the reduction of imines, and such a use does not have any relation to the activity as a TPO agonist which has been found. The EP-A-168,343 describes the use of compounds of general formula I for dyeing plastics. The preparation of compounds of general formula III, wherein Q represents a ligand containing nitrogen such as ammonia or a pyridine derivative has been described in Issled. Khim. Khelatnykh Soedin. 3, (1971).

The compounds of general formula II are prepared by condensation of the corresponding aldehydes or ketones with the corresponding hydrazine derivatives or amine derivatives (e.g., Acta Chem. Scand. 15, 1097 (1961)).

The complex compounds of general formula I and III are obtained in a *per se* known manner by contacting the ligands with the corresponding metal acetates and heating in methanol (Zh. Neorg. Khim. 32, 1158 (1987); Zh. Obshch. Khim. 60, 2549 (1990)).

In the preparation of drugs, the substances of general formula I, II or III are mixed with suitable pharmaceuti-

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cal vehicles, flavoring substances, taste improvers, and colorants and formed into tablets or coated tablets, or suspended in water or oil, e.g., olive oil, with addition of appropriate adjuvants.

The compounds of general formula I, II or III and their salts may be applied in liquid or solid form on the enteral or parenteral route. Water is preferably used as injection medium, containing stabilizers, solubilizers and/or buffers usual in injection solutions. For example, such additives are tartrate or borate buffers, ethanol, dimethyl sulfoxide, chelating agents (such as ethylenediaminetetraacetic acid), high molecular weight polymers (such as liquid polyethylene oxide) for viscosity control, or polyethylene derivatives of sorbitol anhydrides. For example, solid vehicles are starch, lactose, mannitol, methylcellulose, talc, highly disperse silicic acid, higher molecular weight polymers (such as polyethylene glycols). For oral administration, taste improvers and sweeteners may additionally be contained, if desired.

The dosage administered will depend on the age, the TPO level present in the patient, health condition, weight, extent of disease, the type of other treatments possibly conducted at the same time, and the type of effect desired. Conventionally, the daily dose of active compound will be from 0.01 to 5 mg/kg body weight.

In addition to the compounds mentioned in the following examples, those compounds which may be derived by combining all the substituents' meanings mentioned in the claims are preferred in the meaning of the present invention.

The invention will be exemplified by the following examples, without being limited thereto.

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**Example 1:****(2-Hydroxybenzylidene-thiobenzoylhydrazinato)nickel(II) (1)**

1.50 g (5.85 mmol) of N'-(2-hydroxybenzylidene)thiobenzoic acid hydrazide **81** was dissolved in 80 ml of boiling methanol. This solution was stirred into a solution of 1.45 g (5.85 mmol) of nickel(II) acetate tetrahydrate in 80 ml of methanol heated to 60°C. After a few minutes, the reddish-brown metal complex began to precipitate. For complete precipitation, a standing period of 24 hours at room temperature was allowed. The metal complex was subsequently sucked off and washed with methanol and distilled water. After drying at 40°C under vacuum, 1.70 g (94%) of **1** was obtained; m.p.: 261-263°C.

MS (pos. LSIMS sp.) m.w.: 313

Calculated (0.5 H<sub>2</sub>O): C 52.22%, H 3.44%, N 8.70%, Ni 18.2%

Found: C 52.67%, H 3.26%, N 8.82%, Ni 18.4%

**Example 2:****(2-Hydroxybenzylidene-thiobenzoylhydrazinato)zinc(II) (2)**

300 mg (1.17 mmol) of N'-(2-hydroxybenzylidene)thiobenzoic acid hydrazide **81** was dissolved in 20 ml of boiling methanol. This solution was stirred into a solution of 257 mg (1.17 mmol) of zinc(II) acetate dihydrate in 10 ml of methanol heated to 60°C. After a few hours, the reddish-brown metal complex began to precipitate. For complete precipitation, a standing period of 72 hours at room temperature was allowed. The metal complex was subsequently sucked off and washed with methanol and distilled water. After drying at 40°C under vacuum, 350 mg (93%) of **2** was obtained; m.p.: >300°C.

MS (pos. LSIMS sp.) m.w.: 318

Calculated (0.5 H<sub>2</sub>O): C 52.22%, H 3.44%, N 8.70%, Zn 18.2%

Found: C 52.67%, H 3.26%, N 8.82%, Zn 18.4%

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**Example 3:****(2-Hydroxy-5-bromobenzylidene-thiobenzoylhydrazinato)-nickel(II) (3)**

400 mg (1.19 mmol) of N'-(2-hydroxy-5-bromobenzylidene)thiobenzoic acid hydrazide **82** was dissolved in 10 ml of boiling methanol. This solution was stirred into a solution of 297 mg (1.19 mmol) of nickel(II) acetate tetrahydrate in 10 ml of methanol heated to 60°C. After a few minutes, the red-brown metal complex began to precipitate. For complete precipitation, a standing period of 24 hours at room temperature was allowed. The metal complex was subsequently sucked off and washed with methanol and distilled water. After drying at 40°C under vacuum, 290 mg (62%) of **3** was obtained; m.p.: 210-212°C.

MS (pos. LSIMS sp.) m.w.: 319

Calculated: C 42.91%, H 2.31%, Br 20.39%, N 7.15%, Ni 14.0%

Found: C 42.66%, H 2.56%, Br 20.58%, N 6.90%, Ni 13.8%

**Example 4:****(2,4-Dihydroxybenzylidene-benzoylhydrazinato)copper(II) (4)**

0.70 g (2.73 mmol) of N'-(2,4-dihydroxybenzylidene)benzoic acid hydrazide **83** was dissolved in 40 ml of boiling methanol. This solution was stirred into a solution of 0.46 g (2.73 mmol) of copper(II) acetate monohydrate in 20 ml of methanol heated to 60°C. After a few hours, the dark brown metal complex began to precipitate. For complete precipitation, a standing period of 72 hours at room temperature was allowed. The metal complex was subsequently sucked off and washed with methanol and distilled water. After drying at 40°C under vacuum, 0.65 g (75%) of **4** was obtained; m.p.: >310°C.

MS (pos. LSIMS sp.) m.w.: 317

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Calculated: C 52.91%, H 3.17%, N 8.81%, Cu 20.0%

Found: C 52.56%, H 3.21%, N 8.72%, Cu 20.2%

**Example 5:****(2,4-Dihydroxybenzylidene-(4-pyridylcarbonyl)-hydrazinato)-zinc(II) (5)**

0.80 g (3.10 mmol) of N'-(2,4-dihydroxybenzylidene)-pyridine-4-carboxylic acid hydrazide **84** was dissolved in 350 ml of boiling methanol. This solution was stirred into a solution of 0.68 g (3.10 mmol) of zinc(II) acetate dihydrate in 60 ml of methanol heated to 60°C. After a few hours, the dark brown metal complex began to precipitate. For complete precipitation, a standing period of 48 hours at room temperature was allowed. The metal complex was subsequently sucked off and washed with methanol and distilled water. After drying at 40°C under vacuum, 0.88 g (89%) of **5** was obtained; m.p.: >310°C.

MS (pos. LSIMS sp.) m.w.: 320

Calculated (0.5 H<sub>2</sub>O): C 47.37%, H 3.05%, N 12.74%, Zn 19.8%

Found: C 47.91%, H 2.87%, N 12.76%, Zn 18.8%

**Example 6:****(2-Hydroxy-5-bromobenzylidene-thiobenzoylhydrazinato)copper(II) (6)**

0.40 g (1.19 mmol) of N'-(2-hydroxy-5-bromobenzylidene)thiobenzoic acid hydrazide **82** was dissolved in 10 ml of boiling methanol. This solution was stirred into a solution of 0.20 g (1.19 mmol) of copper(II) acetate monohydrate in 10 ml of methanol heated to 60°C. After a few hours, the dark brown metal complex began to precipitate. For complete precipitation, a standing period of 48 hours at room temperature



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was allowed. The metal complex was subsequently sucked off and washed with methanol and distilled water. After drying at 40°C under vacuum, 0.38 g (81%) of 6 was obtained; m.p.: >310°C.

MS (pos. LSIMS sp.) m.w.: 396

Calculated: C 42.38%, H 2.29%, N 7.06%, Cu 16.02%

Found: C 42.29%, H 2.23%, N 6.95%, Cu 15.70%

**Example 7:**

**(2-Hydroxy-4-diethylaminobenzylidene-thiobenzoylhydrazinato) nickel(II) (7)**

1.0 g (3.05 mmol) of N'-(2-hydroxy-4-diethylaminobenzylidene)thiobenzoic acid hydrazide 99 was dissolved in 80 ml of boiling methanol. This solution was stirred into a solution of 0.76 g (3.05 mmol) of nickel(II) acetate in 30 ml of methanol heated to 60°C: After a few hours, the dark brown metal complex began to precipitate. For complete precipitation, a standing period of 72 hours at room temperature was allowed. The metal complex was subsequently sucked off and washed with methanol and distilled water. After drying at 40°C under vacuum, 0.96 g (82%) of 7 was obtained; m.p.: 204-206°C.

MS (pos. LSIMS sp.) m.w.: 384

Calculated: C 56.28%, H 4.99%, N 10.94%, Ni 15.2%

Found: C 56.72%, H 5.48%, N 11.06%, Ni 14.1%

**Example 8:**

**(2-Hydroxybenzylidene-isothiosemicarbazonato)nickel(II) (8)**

0.98 g (5.00 mmol) of N'-(2-hydroxybenzylidene)isothiosemicarbazone 85 was dissolved in 80 ml of boiling methanol. This solution was stirred into a solution of 1.20 g

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(5.00 mmol) of nickel(II) acetate tetrahydrate in 80 ml of methanol heated to 60°C. After a few hours, the dark brown metal complex began to precipitate. For complete precipitation, a standing period of 24 hours at room temperature was allowed. The metal complex was subsequently sucked off and washed with methanol and distilled water. After drying at 40°C under vacuum, 1.10 g (87%) of 8 was obtained; m.p.: >310°C.

MS (pos. LSIMS sp.) m.w.: 251

Calculated (0.5 H<sub>2</sub>O): C 36.82%, H 3.86%, N 16.10%, Ni 22.5%

Found: C 36.86%, H 2.70%, N 16.02%, Ni 21.1%

#### Example 9:

#### (2-Hydroxy-5-bromobenzylidene-isothiosemicarbazonato)copper(II) (9)

1.37 g (5.00 mmol) of N'-(2-hydroxy-5-bromobenzylidene)isothiosemicarbazone 86 was dissolved in 120 ml of boiling methanol. This solution was stirred into a solution of 1.00 g (5.00 mmol) of copper(II) acetate monohydrate in 120 ml of methanol heated to 60°C. After a few hours, the dark brown metal complex began to precipitate. For complete precipitation, a standing period of 24 hours at room temperature was allowed. The metal complex was subsequently sucked off and washed with methanol and distilled water. After drying at 40°C under vacuum, 1.2 g (72%) of 9 was obtained; m.p.: 270-72°C.

MS (pos. LSIMS sp.) m.w.: 336

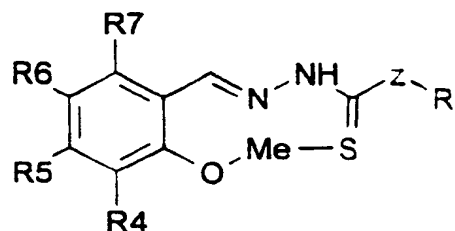
Calculated: C 28.63%, H 1.80%, N 12.52%, Cu 18.93%

Found: C 28.46%, H 1.77%, N 12.24%, Cu 18.30%

Other examples synthesized in an analogous manner (No. 10-29) can be inferred from Table 1. The Examples 1-9 mentioned therein are identical with the Examples above.

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Table 1



Example No.	Comp. No.	Me	Y	Z	R	R <sup>4</sup>	R <sup>5</sup>	R <sup>6</sup>	R <sup>7</sup>
1	1	Ni	S	-	Ph	H	H	H	H
2	2	Zn	S	-	Ph	H	H	H	H
3	3	Ni	S	-	Ph	H	H	Br	H
4	4	Cu	O	-	Ph	H	OH	H	H
5	5	Zn	O	-	Pyridyl-4	H	OH	H	H
6	6	Cu	S	-	Ph	H	H	Br	H
7	7	Ni	S	-	Ph	H	(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> N	H	H
8	8	Ni	S	NH	H	H	H	H	H
9	9	Cu	S	NH	H	H	H	Br	H
10	10	Ni	O	-	Ph	H	H	H	H
11	11	Ni	S	NH	H	H	H	Br	H
12	12	Ni	S	NH	H	OCH <sub>3</sub>	H	H	H
13	13	Ni	O	-	Ph	H	OH	H	H
14	14	Zn	O	-	Ph	H	OH	H	H
15	15	Ni	O	-	4-NH <sub>2</sub> -Ph	H	OH	H	H
16	16	Zn	O	-	4-NH <sub>2</sub> -Ph	H	OH	H	H
17	17	Ni	O	-	4-Pyridyl	H	OH	H	H
18	18	Ni	O	-	Ph	OH	OH	H	H
19	19	Cu	O	-	Ph	OH	OH	H	H
20	20	Cu	O	-	Ph	OCH <sub>3</sub>	H	H	H

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Example No.	Comp. No.	Me	Y	Z	R	R <sup>4</sup>	R <sup>5</sup>	R <sup>6</sup>	R <sup>7</sup>
21	21	Cu	S	-	Ph	H	H	H	H
22	22	Ni	S	-	Ph	OCH <sub>3</sub>	H	H	H
23	23	Ni	S	-	Ph	H	OCH <sub>3</sub>	H	H
24	24	Ni	S	-	Ph	H	H	OCH <sub>3</sub>	H
25	25	Ni	S	-	Ph	H	H	Cl	H
26	26	Ni	S	-	Ph	Cl	H	Cl	H
27	27	Ni	S	-	Ph	Br	H	Br	H
28	28	Ni	S	-	Ph	Br	H	Cl	H
29	29	Ni	S	-	Ph	H	-CH=CH- CH=CH-		H

**Example 30:****Bis(2-hydroxybenzylidene-thiobenzoylhydrazinato)nickel(II) 61**

0.5 g (1.95 mmol) of N'-(2-hydroxybenzylidene)thio-benzoic acid hydrazide **81** was dissolved in 30 ml of boiling methanol. To this solution, a solution of 0.24 g (0.975 mmol) of nickel(II) acetate tetrahydrate in 15 ml of methanol heated to 60°C was added with stirring. After a few minutes, the beige-brown metal complex began to precipitate. For complete precipitation, a standing period of 24 hours at room temperature was allowed. The metal complex was subsequently sucked off and washed with methanol and distilled water. After drying at 40°C under vacuum, 0.5 g (90% of theoretical) of **61** was obtained; m.p.: 243-245°C.

MS (pos. LSIMS sp.) m.w.: 569

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Calculated: C 58.72%, H 3.22%, N 8.95%, Ni 11.20%

Found: C 58.33%, H 3.67%, N 9.96%, Ni 11.30%

**Example 31:**

**Bis(2-hydroxybenzylidene-benzoylhydrazinato)nickel(II) 62**

1.00 g (4.16 mmol) of N'-(2-hydroxybenzylidene)benzoic acid hydrazide **87** was dissolved in 100 ml of boiling methanol. To this solution, a solution of 510 mg (2.08 mmol) of nickel(II) acetate tetrahydrate in 25 ml of methanol heated to 60°C was added with stirring. After a few minutes, the greenish metal complex began to precipitate. For complete precipitation, a standing period of 24 hours at room temperature was allowed. The metal complex was subsequently sucked off and washed with methanol and distilled water. After drying at 40°C under vacuum, 0.98 g (82% of theoretical) of **62** was obtained; m.p.: >300°C.

MS (pos. LSIMS sp.) m.w.: 535

Calculated (0.5H<sub>2</sub>O): C 61.79%, H 3.89%, N 10.29%, Ni 10.74%

Found: C 61.73%, H 4.48%, N 10.19%, Ni 11.00%

**Example 32:**

**Bis(2,3,4-trihydroxybenzylidene-benzoylhydrazinato)nickel(II) 63**

0.70 g (2.57 mmol) of N'-(2,3,4-trihydroxybenzylidene)benzoic acid hydrazide **90** was dissolved in 80 ml of boiling methanol. To this solution, a solution of 0.32 g (1.28 mmol) of nickel(II) acetate tetrahydrate in 25 ml of methanol heated to 60°C was added with stirring. After a few minutes, the brown metal complex began to precipitate. For complete precipitation, a standing period of 24 hours at room temperature was allowed. The metal complex was subsequently

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sucked off and washed with methanol and distilled water. After drying at 40°C under vacuum, 0.46 g (54% of theoretical) of **63** was obtained; m.p.: 210-212°C.

MS (pos. LSIMS sp.) m.w.: 599

Calculated (3H<sub>2</sub>O): C 51.48%, H 4.01%, N 8.58%, Ni 8.99%

Found: C 51.04%, H 3.57%, N 8.47%, Ni 10.00%

**Example 33:**

**(2-Hydroxybenzylidene-thiobenzoylhydrazinato)-pyridinium-nickel(II) 64**

0.50 g (1.2 mmol) of N'-(2-hydroxybenzylidene)thiobenzoic acid hydrazide **81** was dissolved in 80 ml of boiling methanol. This solution was stirred into a solution of 0.48 g (1.2 mmol) of nickel(II) acetate tetrahydrate and 0.16 ml (1.2 mmol) of pyridine in 40 ml of methanol heated to 60°C. After a few hours, the dark brown metal complex began to precipitate. For complete precipitation, a standing period of 48 hours at room temperature was allowed. The metal complex was subsequently sucked off and washed with methanol and distilled water. After drying at 40°C under vacuum, 0.45 g (96%) of **64** was obtained; m.p.: 146-148°C.

MS (pos. LSIMS sp.) m.w.: 391

Calculated: C 58.20%, H 3.86%, N 10.72%, Ni 14.97%

Found: C 58.13%, H 3.58%, N 10.60%, Ni 13.90%

Other examples synthesized in an analogous manner (No. 34-45) can be inferred from Table 2. The Examples 30-33 mentioned therein are identical with the Examples above.

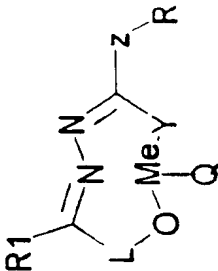


Table 2

Ex. No.	Comp. No.	Me	Y	Z	R	R <sup>1</sup>	L	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	R <sup>5</sup>	R <sup>6</sup>	Q	R <sup>7</sup>
30	61	Ni	S	-	Ph	H	B	-	-	H	H	H	81	H
31	62	Ni	O	-	Ph	H	B	-	-	H	H	H	87	H
32	63	Ni	O	-	Ph	H	B	-	-	OH	OH	H	90	H
33	64	Ni	S	-	Ph	H	B	-	-	H	H	H	Pyridine	H
34	65	Ni	S	-	Ph	H	B	-	-	H	H	H	NH <sub>3</sub>	H
35	66	Cu	S	-	Ph	H	B	-	-	H	H	H	NH <sub>3</sub>	H
36	67	Ni	S	NH	H	H	B	-	-	OCH <sub>3</sub>	H	H	NH <sub>3</sub>	H
37	68	Ni	S	NH	H	H	B	-	-	OCH <sub>3</sub>	H	H	Ph <sub>3</sub> P	H
38	69	Ni	S	NH	H	H	B	-	-	H	H	H	PH <sub>3</sub> P	H

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Ex. No.	Comp. No.	Me	Y	Z	R	R <sup>1</sup>	L	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	R <sup>5</sup>	R <sup>6</sup>	Q	R <sup>7</sup>
39	70	Ni	S	NH	H	H	B	-	-	H	H	H	1-Me-3,4-dihydroisoquinoline	H
40	71	Ni	S	NH	H	H	B	-	-	H	H	H	1,3,3-Tri-me-4-hydro-isoquinoline	H
41	72	Ni	S	Ph	-	H	A	H	CH <sub>3</sub>	-	-	-	NH <sub>3</sub>	-
42	73	Ni	S	Ph	-	H	A	H	Ph	-	-	-	NH <sub>3</sub>	-
43	74	Ni	S	Ph	-	CH <sub>3</sub>	A	H	Ph	-	-	-	NH <sub>3</sub>	-
44	75	Ni	S	NH	H	H	B	-	-	H	-CH=CH-CH=CH-	-CH=CH-CH=CH-	NH <sub>3</sub>	H
45	76	Ni	S	NH	H	H	B	-	-	H	H	NaSO <sub>3</sub>	NH <sub>3</sub>	H



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The compounds of general formula II are prepared by condensation of the corresponding aldehydes or ketones with the corresponding hydrazine derivatives (e.g., Acta Chem. Scand. 15, 1097 (1961)).

**Example 64:****N'-(2-Hydroxy-4-diethylaminobenzylidene)thiobenzoic acid hydrazide (99)**

1.52 g (10 mmol) of thiobenzoic acid hydrazide and 1.93 g (10 mmol) of 2-hydroxy-4-diethylaminobenzaldehyde were added to 80 ml of methanol. This was then heated to boil, and a yellow solution formed. After boiling for 6 hours, the heating was removed and cooling to room temperature was allowed. Upon standing overnight, the substance precipitated in the form of yellow crystals which were sucked off and washed with ice-cold methanol. After drying at 40°C under vacuum, 2.5 g (78%) of **99** was obtained; m.p.: 78-80°C. MS (pos. LSIMS sp.) m.w.: 327

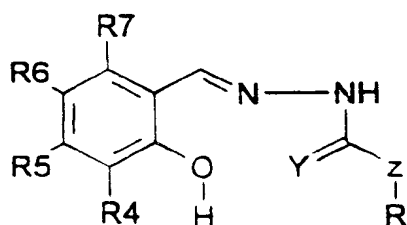
**Example 70:****N'-(2-Hydroxynaphthylidene)thiobenzoic acid hydrazide (105)**

1.52 g (10 mmol) of thiobenzoic acid hydrazide and 1.7 g (10 mmol) of 2-hydroxynaphthaldehyde were added to 50 ml of methanol. This was then heated to boil, and a yellow solution formed. After boiling for 6 hours, the heating was removed and cooling to room temperature was allowed. Upon standing overnight, the substance precipitated in the form of yellow crystals which were sucked off and washed with ice-cold methanol. After drying at 40°C under vacuum, 1.7 g (57%) of **105** was obtained; m.p.: 184-186°C. MS (pos. LSIMS sp.) m.w.: 306

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Other examples synthesized in an analogous manner (No. 46-70) can be inferred from Table 3. The Examples 64 and 70 mentioned therein are identical with the Examples above.

Table 3



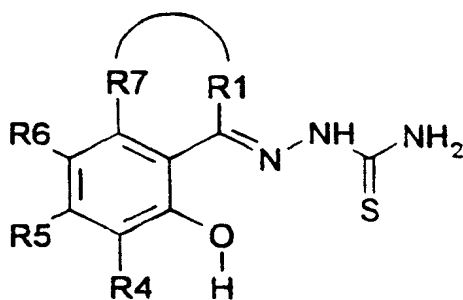
Example No.	Comp. No.	Y	Z	R	R4	R5	R6	R7
46	81	S	-	Ph	H	H	H	H
47	82	S	-	Ph	H	H	Br	H
48	83	O	-	Ph	H	OH	H	H
49	84	O	-	Pyridil-4	H	OH	H	H
50	85	S	NH	H	H	H	H	H
51	86	S	NH	H	H	H	Br	H
52	87	O	-	Ph	H	H	H	H
53	88	S	NH	H	OCH <sub>3</sub>	H	H	H
54	89	O	-	4-NH <sub>2</sub> -Ph	H	OH	H	H
55	90	O	-	Ph	OH	OH	H	H
56	91	O	-	Ph	OCH <sub>3</sub>	H	H	H
57	92	S	-	Ph	OCH <sub>3</sub>	H	H	H
58	93	S	-	Ph	H	OCH <sub>3</sub>	H	H
59	94	S	-	Ph	H	H	OCH <sub>3</sub>	H
60	95	S	-	Ph	H	H	Cl	H
61	96	S	-	Ph	Cl	H	Cl	H

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Example No.	Comp. No.	Y	Z	R	R4	R5	R6	R7
62	97	S	-	Ph	Br	H	Br	H
63	98	S	-	Ph	Br	H	Cl	H
64	99	S	-	Ph	H	(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> -N-	H	H
65	100	S	NH	H	H	H	SO <sub>3</sub> Na	H
66	101	S	NH	H	H	H	2-Me-4,4-Di-Me-pentyl	H
67	102	S	NH	H	tBu	H	tBu	H
68	103	S	NH	H	OMe	H	H	H
69	104	S	NH	H	H	H	NO <sub>2</sub>	H
70	105	S	-	Ph	H	-CH=CH-CH=CH-		H

The following representative compounds are prepared in a way analogous to Examples 46-70.

Table 4



Example No.	Compound No.	R <sup>1</sup> , R <sup>7</sup>	R <sup>4</sup> , R <sup>5</sup> , R <sup>6</sup>
71	106	-CH(CH <sub>3</sub> )-CH <sub>2</sub>	H
72	107		H

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**Pharmacological investigations**

The bioactivity of the compounds according to the invention may be measured using a TPO-dependent cell proliferation test. The substances may not exert any effect on non-transfected parent BaF3 cells. Murine BaF3 cells with IL-3-dependent growth [8] were transfected with human mpl receptor. In the absence of IL-3, proliferation and survival of these cells depend on TPO (Figure 1). The non-transfected parent cell line does not respond to human TPO, yet proliferates in the presence of IL-3. The cell proliferation is determined according to methods well-known in literature (WO 96/40750). The libraries of chemical substances were screened in bioassays using the two cell lines above. The cells were cultivated in the presence of IL-3 (BaF3 parent) and TPO (BaF3 bearing mpl receptor = BaF3/mpl) in RPMI 1640 medium in the presence of 10% FCS (fetal calf serum). For testing, the cells were washed twice in a medium free of IL-3 and TPO, respectively, and resuspended in a medium containing no TPO and IL-3, respectively. The cell suspension was then added in an amount of  $10^4$  cells/well to the wells of a 96 micro-well plate (Costar), which contained TPO or IL-3 and/or the compound. The cells were then incubated in a CO<sub>2</sub> incubator for 48-72 hours at 37°C. The proliferative activity was determined by addition of WST (WST: cell proliferation reagent; BM catalog No. 1644807 "Tetrazolium Salz"). WST is converted to formazan by proliferative cells, and this conversion as a measure for proliferation is determined using the OD (OD: optical density) at 570 nm in an ELISA plate measuring instrument.

To determine the half maximum stimulation, the background (cells with no substance) was subtracted from the maximum signal achieved, and this value was divided by 2. This value plus background value was then used to determine the EC<sub>50</sub> (half maximum excitatory concentration: substrate concentration where the substance has half the maximum activ-

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ity in the BaF3/mpl receptor proliferation test). Table 5 exemplifies the EC<sub>50</sub> values for two tested compounds.

The tested compounds stimulate proliferation of BaF3 cells transfected with mpl receptor in a dosage-dependent fashion. Proliferation of parent cell lines is not stimulated. Even in the absence of TPO, the compounds stimulate proliferation of the BaF3/mpl cells in a culture over weeks.

Table 5

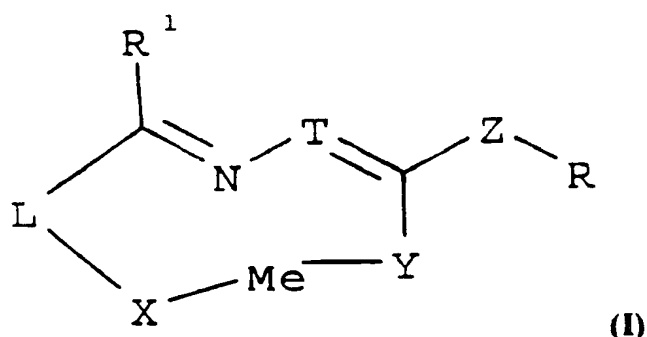
Example No.	EC <sub>50</sub> (µg/ml)
1	0.4
3	0.5

- [1] F.J. de Sauvage et al., *Nature* **1994**, 369, 533-538
- [2] Si Lok et al., *Nature* **1994**, 369, 565-568
- [3] T.D. Bartley et al., *Cell* **1994**, 77, 1117-1124
- [4] N. Methia et al., *Blood* **1993**, 82, 1395-1401
- [5] A. Grossmann et al., *Exp. Hematol.* **1996**, 24, 1238-1246
- [6] R. Basser et al., *Blood* **1997**, 89, 3118-3128
- [7] M. Fanucchi et al., *New Engl. J. Med.* **1997**, 336, 404-409
- [8] R. Palacios et al., *Cell* **1985**, 41, 727-734

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**Claims:**

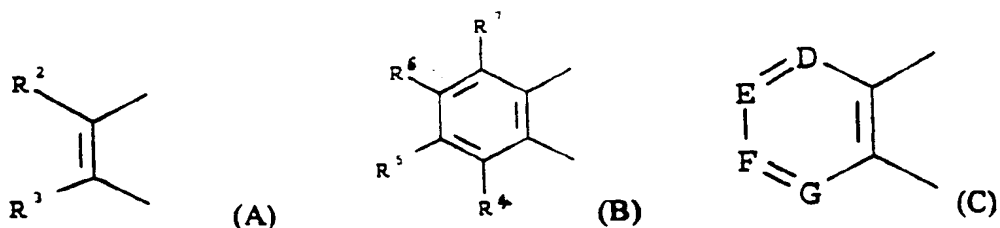
1. Use of metal complexes of general formula I



wherein

- Me represents cobalt, copper, nickel or zinc, which may optionally form a bond to N;
- X represents sulfur, oxygen or an amino group which may be substituted by C<sub>1</sub>-C<sub>10</sub> alkyl or benzyl;
- Y represents oxygen, sulfur or an amino group which may be substituted by C<sub>1</sub>-C<sub>10</sub> alkyl, benzyl or phenyl;
- T represents nitrogen or CR<sup>12</sup>, wherein R<sup>12</sup> may be hydrogen, C<sub>1</sub>-C<sub>10</sub> alkyl, benzyl or phenyl;
- Z represents oxygen, NH or a bond;
- R represents hydrogen, phenyl which may optionally have one or more substitutions, or pyridyl;
- R<sup>1</sup> represents hydrogen, C<sub>1</sub>-C<sub>10</sub> alkyl, phenyl which may optionally have one or more substitutions, a carboxyl or C<sub>1</sub>-C<sub>10</sub> alkoxycarbonyl group;
- L represents an ethylene group (A), an aromatic ring (B), or a heterocyclic ring (C):

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wherein

$R^2$ ,  $R^3$  independently represent hydrogen,  $C_1$ - $C_{10}$  alkyl, phenyl, carboxyl,  $C_1$ - $C_{10}$  alkoxy, carbonyl, or aminocarbonyl;

$R^4$ ,  $R^5$ ,  $R^6$ , and  $R^7$  independently represent hydrogen, chlorine, bromine, iodine, fluorine, trifluoromethyl, cyano,  $SO_3H$ ,  $SO_3Na$ ,  $-SO-R^9$ ,  $-SO_2-R^9$ , nitro, phenyl which may optionally be substituted,  $C_1$ - $C_{10}$  alkyl,  $C_1$ - $C_{10}$  alkoxy,  $C_1$ - $C_{10}$  acyloxy, aralkoxy,  $-CO-R^9$ ,  $NR^{10}R^{11}$ , hydroxy, or cycloalkyl; wherein

$R^9$  may be hydroxy,  $C_1$ - $C_{10}$  alkyl, phenyl, amino, mono- or dialkylamino;

$R^{10}$ ,  $R^{11}$  independently represent hydrogen,  $C_1$ - $C_{10}$  alkyl, phenyl, benzyl, or  $C_1$ - $C_{10}$  acyl;

$R^5$ ,  $R^6$  together represent the  $-CH=CH-CH=CH-$  group;

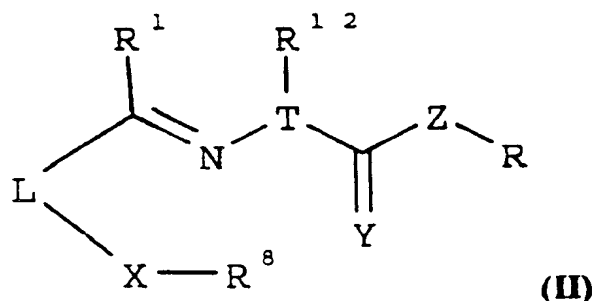
$R^1$ ,  $R^7$  together may form a carbocyclic saturated or unsaturated ring system having 5-14 C atoms, which may optionally have one or more substitutions by halogen, nitro, hydroxy,  $C_1$ - $C_{10}$  alkyl,  $C_1$ - $C_{10}$  alkoxy,  $C_1$ - $C_{10}$  alkoxy, carbonyl, amino, sulfonyl, sulfinyl, mercapto,  $C_1$ - $C_{10}$  alkylmercapto, mono- or di- $C_1$ - $C_{10}$ -alkylamino;

D, E, F, G independently represent  $CR^4$  or N, where either the symbols  $D=E$  or  $F=G$  may also represent oxygen, sulfur or  $NR^{10}$ , or the symbols  $D=E$ ,  $E-F$ ,  $F=G$  may be components of another fused ring system;

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in the production of drugs for treating and preventing thrombopenias and anemias, and their optically active forms, racemates, tautomers, diastereomeric mixtures, as well as the physiologically tolerable salts and prodrugs of these compounds.

2. Use of compounds of general formula II



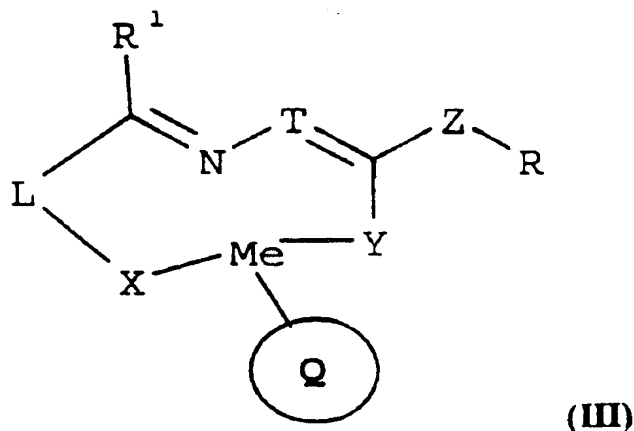
wherein

R, R<sup>1</sup>, R<sup>12</sup>, Y, Z, L, and T have the meanings specified for formula I according to claim 1, X represents sulfur, oxygen or an amino group which may be substituted by C<sub>1</sub>-C<sub>10</sub> alkyl or benzyl, and R<sup>8</sup> represents hydrogen, benzyl, acetyl or C<sub>1</sub>-C<sub>10</sub> alkyl, and their optically active forms, racemates, tautomers, diastereomeric mixtures, as well as the physiologically tolerable salts and prodrugs in the production of drugs for treating and preventing thrombopenias and anemias.



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3. Use of compounds of general formula III



wherein

R, R<sup>1</sup>, L, Me, T, X, Y, and Z have the meanings specified for formula I according to claim 1, Q represents tetrahydrofuran, dimethyl sulfoxide, dimethylformamide, ammonia, a primary, secondary or tertiary amine, pyridine, a trialkylphosphine or triphenylphosphine, 1-Me-3,4-dihydroisoquinoline, 1,3,3-trimethyl-4-hydroisoquinoline, or Q represents a compound of general formula I according to claim 1, or a compound of general formula II according to claim 2, and their optically active forms, racemates, tautomers, diastereomeric mixtures, as well as the physiologically tolerable salts and prodrugs in the production of drugs for treating and preventing thrombopenias and anemias.

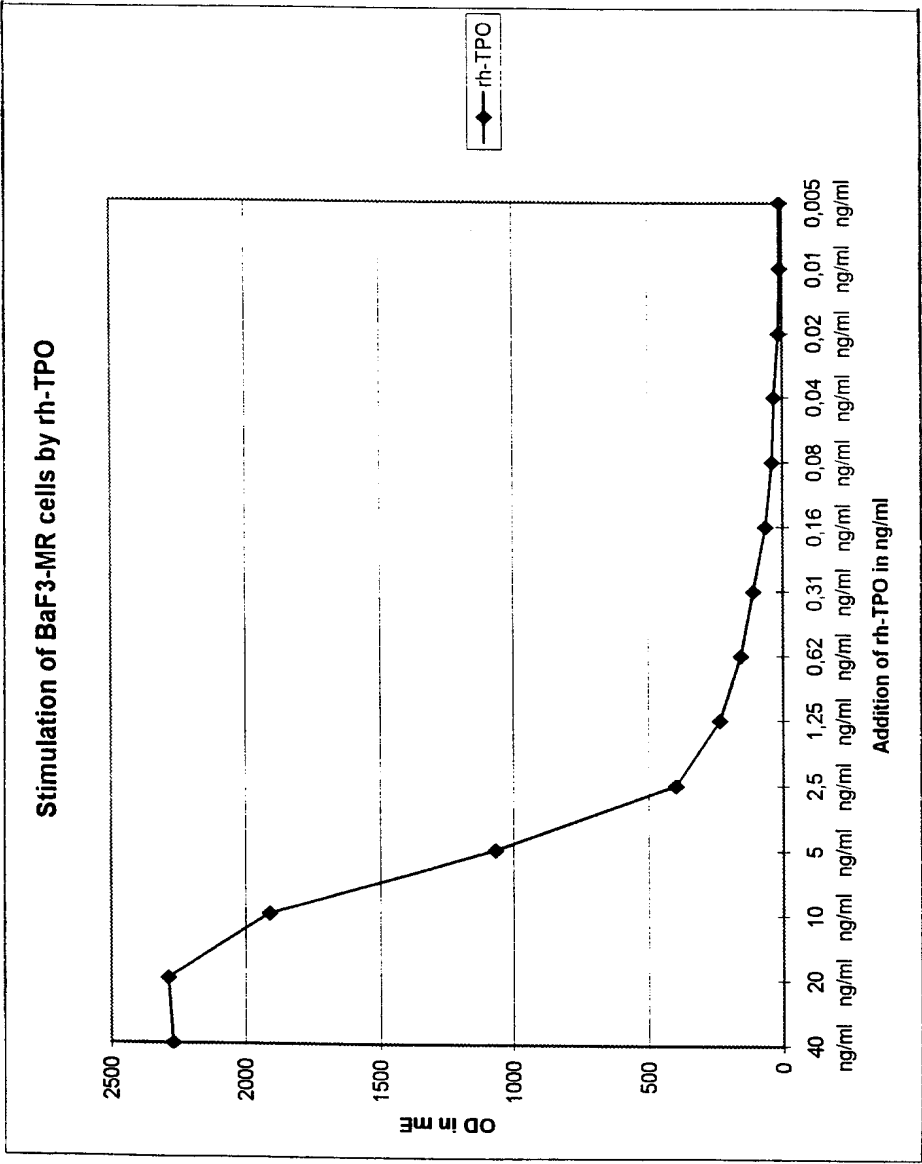
4. Use of compounds of general formula I according to claim 1, of general formula II according to claim 2, or of general formula III according to claim 3 in the production of drugs for the treatment of diseases where thrombopoietin or another protein/peptide binding to the mpl receptor is used as therapeutic agent.
5. Compounds of general formula I according to claim 1, of general formula II according to claim 2, or

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of general formula III according to claim 3, wherein Z is a bond, R is phenyl or pyridyl which optionally may be substituted, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, and R<sup>7</sup> independently represent hydrogen, halogen, amino, mono-C<sub>1</sub>-C<sub>10</sub>-alkylamino, di-C<sub>1</sub>-C<sub>10</sub>-alkylamino, C<sub>1</sub>-C<sub>10</sub> acylamino, or benzyloxy, with the proviso, that R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, and R<sup>7</sup> do not represent hydrogen at the same time, and that R<sup>6</sup> may not be bromine or nitro if R<sup>4</sup>, R<sup>5</sup> and R<sup>7</sup> represent hydrogen at the same time.

6. Drugs, containing at least one compound of general formula I, II or III according to claim 5, and suitable pharmaceutical vehicles and adjuvants.
7. Use of compounds of general formula I, II or III according to claim 5 in the production of drugs for the treatment of diseases, particularly thrombopenias and anemias, where thrombopoietin or another protein/peptide binding to the mpl receptor is used as therapeutic agent.
8. Use of compounds according to claims 1, 2, 3 or 5 in the production of a drug for stimulating platelet formation and stem cell mobilization *in vivo*.
9. Use of compounds according to claims 1, 2, 3 or 5 in the production of a drug for stimulating megakaryocyte and platelet formation *in vitro*.
10. Use of compounds according to claims 1, 2, 3 or 5 in the production of a drug for stimulating erythrocyte formation.
11. Use of compounds according to claims 1, 2, 3 or 5 in the production of a drug for stem cell expansion.

Figure 1



TPO = recombinant human thrombopoietin (RD-Systems Co. (mw: 35 kDA))

# INTERNATIONAL SEARCH REPORT

National Application No  
PCT/EP 98/05492

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K31/44 A61K31/165 A61K31/28 A61K31/30 A61K31/315

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BAKER, E. ET AL: "Evaluation of the iron chelation potential of hydrazones of pyridoxal, salicylaldehyde and 2-hydroxy-1-naphthylaldehyde using the hepatocyte in culture" HEPATOLOGY, vol. 15, no. 3, 1992, pages 492-501, XP000654330	2
A	see the whole document --- -/--	1,3-11

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### ° Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

10 February 1999

Date of mailing of the international search report

17/02/1999

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Mair, J

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 98/05492

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZELENIN, K.N. ET AL: "Synthesis and antimicrobial activity of tridentate thiobenzoylhydrazine-based complexes" KHIM.-FARM. ZH., vol. 24, no. 12, 1990, pages 40-43, XP002067534 see the whole document * insbesondere Verbindungen VIII, IX, X und V *	5,6
X	--- GOTMARE, S. ET AL: "Potentiometric studies on complexes of some bivalent metal ions with Schiff bases of 2,4-dihydroxybenzaldehyde and their biological activities" J. INST. CHEMISTS, vol. 63, no. 5, September 1991, pages 185-186, XP002067535 INDIA see the whole document	5,6
X	--- MOHAN, M. ET AL: "Synthesis, characterization and antitumor activity of iron(II) and iron(III) complexes of 3- and 5-substituted salicylaldehyde benzoyl hydrazones" INORG. CHIM. ACTA, 1987, 135, 167-77, XP002067536 see the whole document	5,6
X	--- EL-SEBAI, A. I. ET AL: "Synthesis of some new acid hydrazides structurally related to certain tuberculostatic agents" EGYPT. J. PHARM. SCI., 1973, 14, 67-73, XP002067537 see the whole document	5,6
X	--- MAURYA, MANNAR R. ET AL: "Dioxotungsten(VI) complexes of ONO donor ligands and the x-ray crystal structure of (W02(o-OC6H4CH:NN:C(O)C6H5)(MeOH)).cntdot. MeOH" BULL. CHEM. SOC. JPN., 1995, 68, 2847-52, XP002067538 * siehe Verbindung nr. 2,4 und 8 *	5
X	--- MAURYA, MANNAR R. ET AL: "Reactivity of bis(acetylacetonato)dinitrosylmolybdenum(0) towards Schiff bases derived from salicylaldehyde or o-vanillin and benzoylhydrazide, or isonicotinoylhydrazide" POLYHEDRON, 1993, 12, 159-63, XP002067539 see the whole document	5
	--- -/--	

## INTERNATIONAL SEARCH REPORT

II International Application No

PCT/EP 98/05492

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BABAIAH, O. ET AL: "Rapid, selective, direct and derivative spectrophotometric determination of titanium with 2,4-dihydroxybenzaldehyde isonicotinoyl hydrazone" TALANTA, 1996, 43, 551-558, XP002067540 see the whole document ---	5
X	SYAMAL, A. ET AL: "Bromate-bromide mixture as a titrimetric reagent for the determination of some ortho-hydroxyaldehydes, acids, hydrazides, Schiff bases and their metal complexes" J. INDIAN CHEM. SOC., 1988, 65, 112-16, XP002067541 see the whole document ---	5
X	KATROLIA, S. P. ET AL: "Studies on antifungal agents: aromatic acid hydrazones of vanillin, veratraldehyde, 5-bromo-vanillin and bourbonal" HIND. ANTIBIOT. BULL., 1989, 31, 65-70, XP002067542 see the whole document ---	5
X	US 4 334 015 A (YARIAN) 8 June 1982 see the whole document ---	5
A	VOYATZAKIS, V.A.E. ET AL: "Influence of metallic ions on the antituberculous activity of isonicotinoyl hydrazones" JOURNAL OF PHARMACEUTICAL SCIENCES, vol. 57, no. 7, July 1968, pages 1255-1257, XP002067543 see the whole document ---	5,6
A	SHARMA, M.P. ET AL: "Synthesis and antimicrobial study of some hydrazone metal complexes" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, INDIA SECTION A, vol. LXI, no. 4, 1991, pages 447-452, XP002067544 see the whole document ---	5,6
A	VALENTOVA, J. ET AL: "Radioprotective activity of N-salicylideneaminoalkanoatocopper(II) complexes" PHARMAZIE, vol. 50, no. 6, 1995, pages 442-443, XP002067545 see the whole document ---	1-11
	--- -/--	

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 98/05492

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>LI, ZHI-LIANG ET AL: "Preliminary screening of non-platinum complexes of Schiff bases as antitumour agents using fluorimetry"</p> <p>SCIENCE IN CHINA, SERIES B, vol. 36, no. 2, February 1993, pages 214-224, XP002067546</p> <p>see the whole document</p> <p style="text-align: center;">---</p>	1-11
A	<p>BHARAMAGOUDAR, T.D. ET AL: "Biological activity of Schiff bases and their metal complexes"</p> <p>CURRNT SCIENCE, vol. 56, no. 17, 5 September 1987, pages 889-890, XP002067547</p> <p>see the whole document</p> <p style="text-align: center;">---</p>	1-11
A	<p>RICHARDSON, D.R. ET AL: "The potential of iron chelators of the pyridoxal isoicotinoyl hydrazone class as effective antiproliferative agents II: The mechanism of action of ligands derived from salicylaldehyde benzoyl hydrazone and 2-hydroxy-1-naphthylaldehyde benzoyl hydrazone"</p> <p>BLOOD, vol. 89, no. 8, 15 April 1997, pages 3025-3038, XP002067548</p> <p>see the whole document</p> <p style="text-align: center;">---</p>	1-11
A	<p>CHATTOPADHYAY, D. ET AL: "Structure of salicylaldehyde thiosemicarbazone"</p> <p>ACTA CRYSTALLOGRAPHICA, vol. 44, no. 6, 1988, pages 1025-1028, XP002067549</p> <p>see the whole document</p> <p style="text-align: center;">---</p>	1-11
A	<p>TSAFACK, A. ET AL: "Mode of action of iron(III) chelators as antimalarials IV. Potentiation of desferal ction by benzoyl and isonicotinoyl hydrazone derivatives"</p> <p>JOURNAL OF LABORATORY AND CLINICAL MEDICINE, vol. 127, no. 6, June 1996, pages 574-582, XP002067550</p> <p>see the whole document</p> <p style="text-align: center;">---</p>	1-11
A	<p>WO 97 16535 A (SANDOZ LTD.) 9 May 1997</p> <p>cited in the application</p> <p>see the whole document</p> <p style="text-align: center;">---</p>	1-11
A	<p>WO 97 26907 A (GENENTECH INC.)</p> <p>31 July 1997</p> <p>see the whole document</p> <p style="text-align: center;">-----</p>	1-11

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 98/05492

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
See FURTHER INFORMATION SHEET PCT/ISA/210
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.



**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

Claims partially searched: 1-11

In view of the large number of compounds which are theoretically defined in the independent claims the search has had to be restricted on economic grounds. The search has been based on those compounds for which examples or pharmacological data has been given and the general idea underlying the application.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

I. International Application No

PCT/EP 98/05492

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 4334015	A	08-06-1982	NONE	
WO 9716535	A	09-05-1997	AU 7495396 A CA 2236263 A EP 0858503 A	22-05-1997 09-05-1997 19-08-1998
WO 9726907	A	31-07-1997	AU 1533597 A CA 2242417 A EP 0876152 A	20-08-1997 31-07-1997 11-11-1998